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Growth Hormone Attenuates Early Left Ventricular Remodeling and Improves Cardiac Function in Rats With Large Myocardial Infarction

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Objectives. **We sought to investigate the cardiac effects of growth hormone (GH) administration during the early phase of pathologic remodeling in a rat model of large myocardial infarction (MI).**

Background. **Recent evidence suggests that exogenous administration of GH evokes a hypertrophic response and increases left ventricular (LV) function in vivo in rats with normal or chronically failing hearts. We hypothesized that these effects would attenuate ventricular remodeling early after MI.**

Methods. **Fifty-eight male rats underwent sham operation (n = 19) or had induced MI (n = 39). The day after the operation, the infarcted rats were randomized to receive 3 weeks of treatment with GH, 3 mg/kg body weight per day (n = 19) or placebo (n = 20). Echocardiography, catheterization and isolated whole heart preparations were used to define cardiac structure and function.**

Results. **Growth hormone caused hypertrophy of the noninfarcted myocardium in a concentric pattern, as noted by higher echocardiographic relative wall thickness at 3 weeks and by morphometric histologic examination. Left ventricular dilation was reduced in the GH-treated versus placebo group (echocardiographic LV diastolic diameter to body weight ratio 2.9 ± 0.1 vs. 3.5 ± 0.2 cm/kg; $p < 0.05$). In vivo and in vitro cardiac function was improved after GH treatment. Despite elevated insulin-like growth factor-1 (IGF-1) serum levels in GH-treated rats, myocardial IGF-1 messenger ribonucleic acid was not different among the three groups, suggesting that an increase in its local expression does not appear necessary to yield the observed effects.**

Conclusions. **These data demonstrate that early treatment of large MI with GH attenuates the early pathologic LV remodeling and improves LV function.**

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Ischemic heart failure is thought to arise directly from the loss of functioning myocardium due to myocardial infarction (MI) and from many secondary processes that contribute to the depression of left ventricular (LV) function. Chief among these is pathologic remodeling, in which dilation and resultant afterload excess combine to initiate a downward spiral of deterioration in function, with subsequent hemodynamic and neurohumoral adaptations to LV dysfunction (1,2). Prevention or attenuation of these secondary processes is an important therapeutic goal.

Recently, several studies have suggested that growth hormone (GH) targets the heart (3–5) and may be of benefit in chronic heart failure (6–8). In particular, two animal studies have shown an improved hemodynamic profile after GH administration in established heart failure due to MI (6,7). These beneficial effects may be transferable to humans: GH administration in seven patients with idiopathic dilated cardiomyopathy resulted in improvement in hemodynamic variables, myocardial energy metabolism and clinical status (8).

These earlier investigations focused on treatment of chronic, well-established heart failure, studied when most of the dynamic remodeling processes, including attendant gene activation, LV dilation and hypertrophy of the noninfarcted myocardium, have already occurred in the rat model used (treatment begun 4 weeks and 3 months after MI) (1). However, in reviewing the known effects of GH on the heart, we postulated that it might also have salutary effects early after MI, by attenuating remodeling and thereby preventing or limiting the development of ischemic heart failure. Growth hormone's ability to induce concentric hypertrophy (9,10) would reduce LV wall stress (11), and its effects on contractility (9,12) would enhance the function of the noninfarcted myocardium, both of which would be of particular benefit if applied while the process of remodeling was beginning or ongoing. Thus, in the current study, GH treatment was initiated 24 h after coronary ligation in a well-established rat model of large MI. A combined histologic, echocardiographic, high fidelity

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Abbreviations and Acronyms

ANOVA = analysis of variance
cDNA = complementary deoxyribonucleic acid
dP/dt = rate of rise of left ventricular pressure
GH = growth hormone
IGF-1 = insulin-like growth factor-1
LV = left ventricular (ventricle)
MI = myocardial infarction
mRNA = messenger ribonucleic acid

micromanometric and isolated whole heart approach was used to define changes in structure and in vivo and in vitro function; the paracrine/autocrine effects of GH were examined assessing ventricular expression of insulin-like growth factor-1 (IGF-1) messenger ribonucleic acid (mRNA).

Methods

All animal procedures were conducted in accordance with the requirements of the American Physiological Society, and confirm to the "Position of the American Heart Association on Research Animal Use" adopted by the Association in November 1984. Myocardial infarction was induced according to previously described methods (13). Briefly, male Sprague-Dawley rats (Charles River Breeding Laboratories) with a body weight ranging from 220 to 250 g, were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight) and orally intubated. After performing an anterior thoracotomy, the heart was exteriorized and a 6-0 silk suture was snugly placed around the proximal left anterior descending coronary artery. Nineteen sham animals (sham group) underwent the same operation, but did not receive the ligation of the coronary artery. The perioperative mortality rate in the MI group was ~50%. The day after the procedure all surviving animals were screened by transthoracic echocardiography (see later) for the presence of large infarctions involving at least 35% of the left ventricle; four rats were excluded because of smaller infarct sizes. On the same postoperative day, after the echocardiogram, the animals were randomized to receive GH (n = 19; 3 mg/kg per day of recombinant human GH through two daily subcutaneous injections; MI-GH group) or placebo (n = 20; MI group) for 3 weeks. This treatment protocol was designed based on our previous observations of a significant hypertrophic response in vivo and increased cardiac function in normal rats given a similar dose of GH for a similar treatment period (9). The majority of the myocardial remodeling process in the rat (70% to 80%) is complete within 3 weeks (1), and the potential development of antibodies to human GH with longer treatment is avoided. Recombinant human GH was provided by Genentech, Inc.

Echocardiography. Transthoracic echocardiography was performed in all animals before the operation (baseline echocardiogram), 1 day after the operation (to ensure large MI in all animals subsequently studied) and after 3 weeks of therapy.

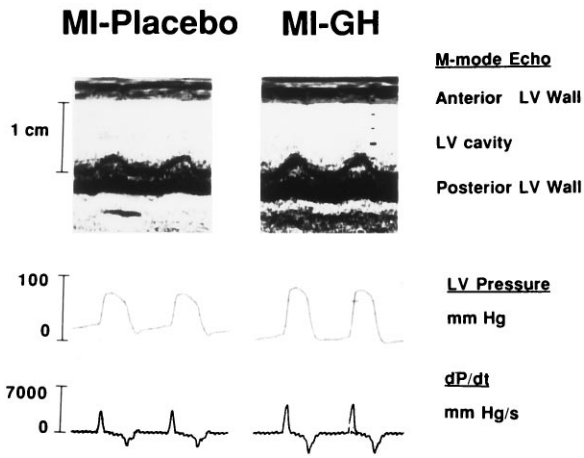

Figure 1. Representative echocardiographic (**top panels**) and hemodynamic (**bottom panels**) tracings obtained from rats given placebo after myocardial infarction (MI-placebo) and rats treated with growth hormone after myocardial infarction (MI-GH). Growth hormone–treated animals showed hypertrophy and increased systolic thickening of the noninfarcted posterior wall. Left ventricular end-diastolic pressure was significantly lower and peak positive and peak negative rate of rise of LV pressure (dP/dt) significantly higher after GH treatment. Heart rate was similar in both tracings.

Previous reports from our laboratory (9,13) and that of others (14) have demonstrated the accuracy and reproducibility of transthoracic echocardiography in rats. Briefly, rats were anesthetized with a combination of ketamine hydrochloride 50 mg/kg (Parke Davis) and xylazine 10 mg/kg intraperitoneally (Lloyd Laboratories) and placed on a specially designed apparatus. Echocardiography was performed from underneath with a Hewlett-Packard Sonos 1500 sector scanner equipped with a 7.5-MHz phased-array transducer. Two-dimensionally guided M-mode tracings were recorded with a strip-chart recorder at a paper speed of 100 mm/s (Fig. 1). Posterior wall thickness and LV internal dimensions were measured according to the leading edge method of the American Society of Echocardiography (15). Left ventricular outflow tract diameter was measured on a still-frame two-dimensional image at the base of the aortic leaflets in a parasternal long-axis view. Infarct size was measured from the 3-week echocardiogram by observing the akinetic region in real time and measuring the percentage of the LV endocardial circumference that was akinetic on a freeze-frame image at end-diastole, as previously described (13). All measurements were performed by one observer who had no knowledge of the previous results and were based on the average of three consecutive cardiac cycles.

Two-dimensionally guided pulsed Doppler recordings of LV inflow were obtained from the apical four-chamber view. Doppler recordings were made at a paper speed of 100 mm/s and analyzed off-line. Measurements were made from six consecutive cycles to minimize beat to beat variability. Maximal early and late diastolic flow velocities were derived from mitral inflow velocities, and LV outflow tract velocimetry was recorded from a five-chamber view. Stroke volume was calculated as:

$$\text{Aortic velocity time integral} \times (\pi[\text{LV outflow tract}/2]^2),$$

and multiplied by heart rate to calculate cardiac output. When appropriate, structural and functional indexes were normalized to body weight or tibial length, or both, because of the considerable somatic growth in the MI-GH group.

Hemodynamic studies. Within 12 h of the final echocardiogram, the rats were anesthetized with ketamine and xylazine at the same doses used for the earlier echocardiograms. A calibrated 2F micromanometer-tipped catheter (Millar Instruments) was passed through the carotid artery into the LV under constant pressure monitoring. Left ventricular end-diastolic pressure was recorded with an expanded scale and rate of rise of LV pressure (dP/dt) was obtained from a differentiating circuit in the physiologic recorder (model 2400, Gould, Inc.). Because changes in LV shape and uniformity after MI prevent the calculation of true LV wall stress from monodimensional images, we devised an approximate measure of load of the noninfarcted myocardium, termed *posterior wall load index*, using the following formula (16):

$$0.334 \times \text{LV pressure} \times (\text{LVID}/[1 + \text{PWT}/\text{LVID}]),$$

where LVID is left ventricular internal diameter (end-systolic or end-diastolic), and PWT is posterior wall thickness. Although pressures and dimensions were measured under identical anesthetic conditions, they were not performed simultaneously. Nevertheless, we believe that additional useful information could be derived from these data. An estimation of vascular resistance was calculated as:

$$\text{Mean arterial blood pressure}/\text{Cardiac output},$$

and normalized to body weight.

Isolated whole heart preparation. An additional subgroup of 18 rats (six from each group) was killed, and the isolated hearts were placed in an isovolumic, buffer-perfused rat heart preparation according to the Langendorff technique, as previously described (12). After anesthetization, the hearts were quickly removed and put into ice-cold Krebs-Henseleit solution (see later), weighed and mounted on a cannula inserted into the ascending aorta. Retrograde aortic perfusion of the coronary arteries was performed within 30 s through a constant flow of 10 ml/min per g heart weight, and the pressure monitored by a Statham P23Db transducer. Coronary flow in the MI group was corrected for scar weight (16% of total heart weight on average, assuming that perfusion of the scar is minimal) (17,18). The composition of the perfusate was as follows (mmol/liter): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 23 and dextrose 5.5, saturated with a 95% oxygen/5% carbon dioxide gas mixture to a pH of 7.4 ± 0.2. Left ventricular pressure was measured using a fluid-filled latex balloon inserted through the mitral valve. After 15 to 30 min at 25°C, the temperature was gradually increased to 37°C and the hearts were paced at 4 Hz. Measurements of LV function were obtained when the preparation achieved a steady state after instrumentation (~15 min) at a common diastolic pressure of 10 mm Hg. Both absolute values of developed pressure and those normalized to LV wet weight were assessed.

Postmortem studies. After catheterization, the animals were randomly assigned to either RNA extraction and dot-blot analysis (seven each from the MI and sham groups, and six from the MI-GH group) or histologic examination (six from each group). A subgroup of 18 animals (six per each treatment group) underwent echocardiography and isolated whole heart studies. Blood samples and tibial length measurements were obtained from all animals.

Blood analysis. Duplicate hematocrit samples were prepared in microhematocrit tubes. Serum was frozen at −20°C for subsequent analysis. Human and rat GH was measured in rat serum by enzyme-linked immunosorbent assay, and total serum IGF-1 was measured by radioimmunoassay, according to previously described methods (19,20).

Ribonucleic acid extraction and dot-blot analysis. Because the effects of GH appear to be mediated in part by local production of IGF-1 in target tissues (14), we measured the ventricular level of IGF-1 mRNA by dot blotting. Immediately after catheterization, the hearts were excised and the atria trimmed, and the ventricular tissue was quickly dropped into liquid nitrogen and stored at −70°C for subsequent analysis. Total muscle RNA was extracted by the guanidinium thiocyanate-phenol-chloroform method (22). A 40-μg aliquot of total RNA per sample was denaturated and applied to nylon membranes (Gene Screen Plus, Dupont, NEN Products) using a dot-blot vacuum manifold apparatus (Scheider and Schuell). After immobilization by ultraviolet cross-linking, the blots were hybridized overnight at 42°C with phosphorus-32–labeled IGF-1 complement deoxyribonucleic acid (cDNA) (Dr. P. Rotwein, Washington University) or 18S ribosomal cDNA (Dr. P. Bachierre, Centre de Recerche de Biochimie et Genetique Cellulaires, Toulouse Cedex, France) and washed according to methods described by the manufacturer of the nylon membranes, and the intensity of labeling of individual dots was quantitated with a Phosphorimager (Molecular Dynamics). The 18S ribosomal cDNA probe was used to correct for total RNA loading differences.

Histologic examination. Left ventricles were immersion fixed in 10% buffered formalin. Four cross sections, cut from apex to base, were embedded in paraffin and stained with hematoxylin-eosin for measurements of muscle fiber diameter and with Masson trichrome for assessment of interstitial fibrosis. The four sections were then projected, and average infarct size was estimated by measuring the percentage of the total endocardial circumference replaced by scar tissue. Quantitative evaluation of myocyte hypertrophy was carried out by morphometric analysis, according to previously described methods (23), by an observer who had no knowledge of the study protocol, on tissue blocks obtained from the noninfarcted interventricular septum. Briefly, each section was projected by using a binocular microscope (Zeiss, Germany) attached to a video camera at ×400 magnification and a personal computer (Apple Computer) equipped with morphometric software. The circumferences of 100 myocytes per

Table 1. Animal Characteristics and Hormone Determinations

	Sham Group	MI Group	MI-GH Group
Body weight (g)			
Baseline	245 ± 5	247 ±4	255 ± 3
3 Weeks	273 ± 4*	258 ± 6	330 ± 4*†‡
Tibial length (mm)	38.6 ± 0.3	38.9 ± 0.4	40.9 ± 0.3†‡
Rat GH serum levels (ng/ml)	0.86 ± 0.2	0.7 ± 0.3	1.1 ± 0.4
Human GH serum levels (ng/ml)	Undetectable	Undetectable	2,000 ± 220
IGF-1 serum levels (ng/ml)	292 ± 13	286 ± 18	390 ± 25†‡
IGF-1 mRNA ventricular levels (arbitrary units)	100 ± 3	96 ± 3	109 ± 10
Hematocrit (ml/dl)	51 ± 10	50 ± 10	49 ± 10

*$p < 0.05$ versus baseline. †$p < 0.05$ versus sham group. ‡$p < 0.05$ versus myocardial infarction group. Data are presented as mean value ± SEM. GH = growth hormone; IGF-1 = insulin-like growth factor-1; MI = placebo-treated group with myocardial infarction; MI-GH = MI group treated with GH; mRNA = messenger ribonucleic acid.

animal were digitized on each of the four sections, and average myocyte area was calculated. Quantitative assessment of interstitial tissue as a measure of fibrosis was accomplished with a special grid on which horizontal and vertical lines provided 100 intersecting points, at ×400 magnification. Four fields on each of the four sample sites were examined for each animal, yielding a total of 16 fields in each rat. Reproducibility studies showed a good correlation between data obtained from two studies in four rats, for both techniques ($r = 0.90$).

Tibial length. At the end of the experiment, the right hind legs of the rats were removed by disarticulating the femurs from the acetabula at the hip. The tibias were dissected free of soft tissue and frozen at −20°C. Four radiographic films (X-Omat XTL2, Eastman Kodak Company) of the tibias were then obtained, and the tibial length of each animal was measured on the radiograph with a caliper.

Statistical analysis. All data are given as mean value ± SEM. Statistical analysis was performed using a Sun Microsystem Station equipped with the PROPHET software package. Intergroup comparisons of echocardiographic indexes were performed using a two-way analysis of variance (ANOVA) with repeated measures in one factor (time), followed by the Neumann-Keuls test. One-way ANOVA was used for the other comparisons, also followed by the Neumann-Keuls test. A value of $p < 0.05$ was considered significant.

Results

Myocardial infarction in the absence of GH treatment resulted in a lower average weight gain compared with the sham group (4% vs. 11%). The MI-GH group exhibited a weight gain of ~30% (Table 1), which was significantly greater than that in the sham-operated control animals. Body weight increase in the GH group followed a linear pattern (8% first week, 9% second week and 13% third week vs. baseline values), thereby demonstrating a continued effect of GH over time. Tibial length was significantly increased in the GH-treated animals as compared with the untreated rats. Hematocrit was not different among the three groups.

Rat GH levels were similar in all groups and, as expected, human GH levels were undetectable in the sham and MI groups and increased to ~2,000 ng/ml in GH-treated animals (Table 1). Insulin-like growth factor-1 serum levels were also significantly higher in the MI-GH group by ~30% to 35%. Quantitation of total RNA by dot blotting showed no differences among the three study groups, when determined either with or without correction for loading by measuring 18S RNA.

Histologic and echocardiographic infarct sizes were not different between the untreated MI and MI-GH groups (Table 2). There was no evidence of fibrosis in either MI group.

Compared with the sham group, the untreated MI group showed LV dilation, increased posterior wall thickness and reduced systolic thickening of the noninfarcted posterior wall, with a reduction of relative wall thickness and decreased indexes of cardiac performance (Tables 3 and 4). Growth hormone treatment oriented ventricular remodeling toward greater functional advantage: there was significantly less cavity dilation, less reduction in relative wall thickness and improvement of all echocardiographic functional indexes (Fig. 1, Tables 3 and 4). In vivo hemodynamic data substantiated the beneficial effects of GH treatment on cardiac function: systolic function was improved, as demonstrated by increased peak positive dP/dt and by a blunted reduction of systolic and mean arterial pressure after MI; diastolic function improved, as shown by lower filling pressures and higher peak negative dP/dt in the GH-treated group compared with the MI group (Table 5). Because of the combined effects of GH treatment on LV geometry and pressures, systolic and especially diastolic load indexes of the noninfarcted LV posterior wall were both significantly reduced in the MI-GH group compared with the untreated MI group. The difference between the two infarcted groups averaged 15% for systolic and 61% for diastolic load index. An index of vascular resistance was slightly lower in treated rats. At a common diastolic pressure of 10 mm Hg, infarcted animals showed significantly lower values of developed pressure in the isolated whole heart than shams (Table 5). Furthermore, GH-treated animals had significantly higher

Table 2. Measures of Cardiac Tissue Response

	Sham Group	MI Group	MI-GH Group
Infarct size, by histology (%)	—	47 ± 3	48 ± 4
Infarct size, by echocardiography (%)	—	47 ± 3	46 ± 3
Myocyte area (μm^2)	201 ± 12	210 ± 11	258 ± 16†‡
Interstitial tissue (%)	5.0 ± 0.3	4.7 ± 0.2	5.3 ± 0.3
LV wet weight (g)	0.67 ± 0.02	0.70 ± 0.06	0.75 ± 0.08
LV wet/body weight (mg/g)	2.42 ± 0.10	2.67 ± 0.30	2.25 ± 0.20
LV wet/tibial length (mg/mm)	1.73 ± 0.04	1.80 ± 0.07	1.83 ± 0.11
RV wet weight (g)	0.18 ± 0.02	0.32 ± 0.04†	0.33 ± 0.03†
RV wet/body weight (mg/g)	0.66 ± 0.02	1.24 ± 0.05†	1.04 ± 0.04†‡
RV wet/tibial length (mg/mm)	0.47 ± 0.01	0.82 ± 0.02†	0.80 ± 0.01†

*$p < 0.05$ versus baseline. †$p < 0.05$ versus sham group. ‡$p < 0.05$ versus myocardial infarction group. Data are presented as mean value ± SEM. LV = left ventricular; MI = placebo-treated group with myocardial infarction; MI-GH = MI group treated with growth hormone; RV = right ventricular.

developed pressures than the untreated infarcted group, suggesting that increased contractility of the noninfarcted LV wall played an important role in the overall improved hemodynamic profile documented in vivo. Although balloon volumes tended to be higher in the infarcted groups, there were no significant differences among the three groups (0.25 ± 0.11, 0.36 ± 0.12 and 0.35 ± 0.9 ml in controls, placebo infarcted and GH infarcted, respectively).

Discussion

This study demonstrates several beneficial effects of GH treatment on the remodeling process in the well-established model of large MI in the rat. There was a reduction of LV dilation and an improvement of systolic and diastolic function. Hypertrophy of the noninfarcted myocardium with GH was documented both by histologic measures of myocyte area and

Table 3. Echocardiographic Data: Morphology

	Sham Group	MI Group	MI-GH Group
AW diastole (mm)			
Baseline	1.4 ± 0.05	1.3 ± 0.02	1.3 ± 0.01
3 Weeks	1.4 ± 0.04	1.2 ± 0.03†	1.2 ± 0.03†
AW thickening (%)			
Baseline	90 ± 10	88 ± 9	85 ± 11
3 Weeks	85 ± 11	9 ± 8*†	8 ± 8*†
PW diastole (mm)			
Baseline	1.4 ± 0.04	1.4 ± 0.06	1.4 ± 0.05
3 Weeks	1.4 ± 0.04	1.6 ± 0.03*†	1.9 ± 0.01*†‡
PW thickening (%)			
Baseline	82 ± 8	90 ± 7	85 ± 7
3 Weeks	78 ± 9	42 ± 8*†	61 ± 6*†‡
LV diastolic diameter (mm)			
Baseline	6.7 ± 0.2	7.1 ± 0.1	6.8 ± 0.3
3 Weeks	6.8 ± 0.01	9.2 ± 0.4*†	9.3 ± 0.5*†
LV diastolic diameter/BW (cm/kg)			
Baseline	2.6 ± 0.1	2.8 ± 0.3	2.6 ± 0.2
3 Weeks	2.4 ± 0.1	3.5 ± 0.2*†	2.9 ± 0.1*†‡
LV systolic diameter (mm)			
Baseline	3.8 ± 0.2	3.9 ± 0.2	3.7 ± 0.4
3 Weeks	3.7 ± 0.2	7.3 ± 0.3*†	6.8 ± 0.2*†
LV systolic diameter/BW (cm/kg)			
Baseline	1.53 ± 0.1	1.57 ± 0.2	1.46 ± 0.1
3 Weeks	1.35 ± 0.1	2.78 ± 0.3*†	2.05 ± 0.2*†‡
Relative wall thickness			
Baseline	0.43 ± 0.02	0.41 ± 0.01	0.41 ± 0.01
3 Weeks	0.43 ± 0.01	0.34 ± 0.01*†	0.38 ± 0.01*†‡

*$p < 0.05$ versus baseline. †$p < 0.05$ versus sham group. ‡$p < 0.05$ versus myocardial infarction group. Data are presented as mean value ± SEM. AW = anterior wall; BW = body weight; LV = left ventricular; MI = placebo-treated group with myocardial infarction; MI-GH = MI group treated with growth hormone; PW = posterior wall.

Table 4. Echocardiographic Data: Function

	Sham Group	MI Group	MI-GH Group
Heart rate (beats/min)			
Baseline	248 ± 9	260 ± 14	255 ± 9
3 Weeks	246 ± 6	249 ± 9	250 ± 9
Stroke volume (ml)			
Baseline	0.26 ± 0.03	0.27 ± 0.03	0.25 ± 0.03
3 Weeks	0.28 ± 0.02	0.19 ± 0.01*†	0.29 ± 0.02‡
Stroke volume/BW (ml/kg)			
Baseline	1.05 ± 0.03	1.09 ± 0.03	0.98 ± 0.04
3 Weeks	1.03 ± 0.04	0.72 ± 0.03*†	0.89 ± 0.03*†‡
Stroke volume/TL, 3 weeks (ml/dm)	0.72 ± 0.02	0.49 ± 0.04†	0.71 ± 0.04‡
LV fractional shortening (%)			
Baseline	43 ± 3	45 ± 2	45 ± 3
3 Weeks	44 ± 2	19 ± 3*†	28 ± 2*†‡
Cardiac output (ml/min)			
Baseline	65 ± 5	70 ± 4	64 ± 5
3 Weeks	69 ± 6	48 ± 5*†	73 ± 4‡
Cardiac output/BW (ml/kg)			
Baseline	257 ± 10	278 ± 13	250 ± 12
3 Weeks	255 ± 8	185 ± 11*†	220 ± 13†‡
Cardiac output/TL, 3 weeks (ml/dm)	177 ± 7	122 ± 8†	178 ± 9‡
Mitral-E/A ratio			
Baseline	2.2 ± 0.16	2.1 ± 0.14	2.0 ± 0.14
3 Weeks	2.4 ± 0.18	13.1 ± 2*†	6.6 ± 1*†‡

*$p < 0.05$ versus baseline. †$p < 0.05$ versus sham group. ‡$p < 0.05$ versus myocardial infarction group. Data are presented as mean value ± SEM. E/A = early transmitral flow velocity to atrial flow velocity ratio; TL = tibial length. Other abbreviations as in Table 3.

by echocardiographic assessment of posterior wall thickness. These changes, combined with smaller cavity sizes and lower filling pressures, contributed to a reduced systolic and diastolic load index of the noninfarcted posterior wall. An index of vascular resistance was reduced. The results of the isolated whole heart studies strongly suggest that a direct effect on contractility also played a role in improving the hemodynamic profile in vivo, independent of the loading conditions. The dot-blotting data suggest that paracrine/autocrine IGF-1 actions are not important in mediating GH effects in this model system.

Growth hormone and postinfarction ventricular remodeling. We have recently demonstrated that the pathologic postinfarction remodeling process can be tracked longitudinally with transthoracic echocardiography in a rat model of large MI showing: 1) progressive LV cavity dilation; 2) hypertrophy of noninfarcted myocardium; 3) gradual development of regional contractile dysfunction in surviving regions; and 4) abnormalities of diastolic filling (13). The current study shows that GH treatment has beneficial effects on every aspect of this process. We propose that the following physiologic mechanisms are at play:

1. Induction of additional hypertrophy of the noninfarcted myocardium. This represents a means by which LV wall stress can be reduced according to Laplace's law (11,24), and previous clinical and molecular biology studies performed in humans and animals provide a solid background for this "trophic" effect (3–5).

2. Reduced vascular resistance. This is another important mechanism of load reduction and is in keeping with our previous observations in normal rats (9) and normal humans (25,26) subjected to GH excess, and to other reports in experimental heart failure in rats (6,7). Although the mechanism is still unclear, this vasodilatory effect might be mediated by IGF-1, because IGF-1 has been shown to have the same effect in the human forearm (27). Alternatively, a decrease in adrenergic-mediated vasoconstriction due to the better overall hemodynamic profile induced by GH treatment could in part explain this finding.

3. Enhanced function of the surviving myocardium. The hypothesis that GH can directly affect cardiac contractility is supported by several in vitro studies documenting that GH excess increases myofilament calcium sensitivity (12,28).

Table 5. In Vivo Hemodynamic and In Vitro Whole-Heart Indexes of Cardiac Function

	Sham Group	MI Group	MI-GH Group
LV systolic blood pressure (mm Hg)	115 ± 3	72 ± 5*	80 ± 4*
Mean aortic blood pressure (mm Hg)	95 ± 3	66 ± 4*	74 ± 4*
LV end-diastolic pressure (mm Hg)	3.0 ± 0.2	24 ± 2*	11 ± 2*†
Peak positive dP/dt (mm Hg/s)	7,900 ± 340	3,000 ± 120*	5,500 ± 140*†
Peak negative dP/dt (mm Hg/s)	5,800 ± 290	2,300 ± 200*	3,100 ± 300*†
Systemic vascular resistance (mm Hg/ml per min per kg body weight)	0.38 ± 0.01	0.37 ± 0.01	0.32 ± 0.02*†
Posterior wall systolic load index (kdyne/cm^2)	80 ± 5	101 ± 7*	86 ± 4†
Posterior wall diastolic load index (kdyne/cm^2)	5.6 ± 2	54 ± 5*	21 ± 4*†
Developed pressure in vitro (mm Hg)	108 ± 8	54 ± 5*	70 ± 7*†
Normalized developed pressure in vitro (mm Hg/g)	163 ± 13	77 ± 8*	93 ± 6*†
Coronary perfusion pressure in vitro (mm Hg)	77 ± 4	98 ± 4*	93 ± 3*

*$p < 0.05$ versus sham group. †$p < 0.05$ versus myocardial infarction group. Data are presented as mean value ± SEM. dP/dt = rate of rise of left ventricular pressure. Other abbreviations as in Table 3.

4. Beneficial effects of GH treatment on diastolic filling. Reduced LV filling pressures, documented by noninvasive and invasive techniques, are in keeping with our previous in vivo observation that relaxation, as assessed by tau, is enhanced after GH treatment in normal rats (9) and by the reduction of the Doppler-detected isovolumic relaxation time observed in patients with heart failure treated with GH (8). It is conceivable that enhancement of systolic performance by GH might facilitate LV emptying and thereby reduce LV filling pressures (8,9). Alternatively, a direct effect of GH on relaxation or on LV stiffness, or both, is also possible. Further research is needed to clarify this issue.

Taken together, the combined and synergistic effects of GH on myocardial growth, contractility and loading conditions are precisely those which would be predicted to be of benefit given our current understanding of the early remodeling process.

Growth hormone–induced hypertrophy. Although the hypertrophy produced by GH treatment could be detrimental, the preservation of capillary density (29), lack of fibrosis and improvement in diastolic filling (present study, 8,9,30,31) all suggest that GH excess results in a more physiologic rather than pathologic form of hypertrophy. However, this and previous studies (6) concur that GH does not increase overall LV weight in this MI model. This apparent paradox may be explained by the balance between the prevention of LV dilation and the induction of posterior wall hypertrophy.

To date, few studies have addressed whether additional hypertrophy can be beneficial in the setting of postinfarction remodeling. Litwin et al. (32) demonstrated beneficial effects of LV hypertrophy induced by an inhibitor of long-chain fatty acid oxidation or by L-thyroxine (33), although both therapies have little clinical applicability.

A report by Duerr et al. (34) recently found that IGF-1, given in the early phase of postinfarction remodeling in the rat, induced a hypertrophic response and increased stroke volume (normalized to tibial length). However, ejection fraction did not change, and LV diastolic volumes increased after IGF-1 treatment when compared with no treatment. Although Duerr et al. (34) did not examine cardiac geometry in vivo, it is likely that the modification of remodeling was less striking with IGF-1 than that observed with GH in our study because of intrinsic differences in the actions of these two hormones (9,21). In fact, in addition to opposite effects on glucose metabolism, the hormones have differential effects in several peripheral tissues, including the heart (9). Cardiac volumes tended to be lower after GH rather than higher as reported after IGF-1 (9,34), an important advantage of GH in reducing load and attenuating remodeling. Furthermore, in the study by Duerr et al., the rats' smaller average MI size (26% vs. 47% in our study) and the initiation of treatment on the second day after infarction (rather than on the first day) might both have contributed to the lesser effects of IGF-1.

Other investigators have confirmed a beneficial effect of GH in the chronic phase of ischemic heart failure. Yang et al. (6) reported that GH reduced LV filling pressures and peripheral vascular resistance and increased cardiac index in rats 4 weeks after MI. Jin et al. (7), using rats 3 months after MI, demonstrated that the beneficial hemodynamic effects of combined administration of GH and IGF-1 were additive to those induced by captopril, although captopril's effects in reducing LV weight were still evident (7). The design for these studies differs from the present one, as they (6,7) attempted to modify function in well-established heart failure and not the early remodeling process. Further, no in vivo assessment of LV geometry, or in vitro assessment of function, was provided by these earlier studies.

Growth hormone effects on serum and cardiac IGF-1 levels. Although serum IGF-1 levels were significantly increased after 3 weeks of GH treatment, ventricular levels of IGF-1 mRNA were not different among the three study groups. Thus, increased local production of IGF-1 does not appear to have a role in the observed cardiovascular effects of GH. In this model system, IGF-1 may function predominantly as an endocrine hormone rather than as a paracrine or autocrine growth factor, a hypothesis supported by previous studies (35). Alternatively, it is also conceivable that, given the presence of GH receptors in the heart (36) and the demonstration of direct GH effects on several tissues such as the growth plate (37), the reported cardiovascular effects might be accounted for by direct cardiac actions of GH. Because IGF-1 and IGF-1 receptor mRNA have recently been demonstrated to be upregulated in viable right and left ventricular myocytes 2 days after MI (38), it is possible that local IGF-1 production has an important role early in the post-MI period and then diminishes in the late phase of remodeling.

Study limitations. Data are presented unnormalized as well as normalized to both body weight and tibial length. This was done because it has been suggested that tibial length is more related to lean body mass than to total body weight (39). We (9) and others (6,34) have used these normalization methods in similar model systems.

Although it is not ideal to assess pressures under anesthesia, all animal groups were consistently handled, thereby supporting the validity of intergroup differences. Similarly, in devising a posterior wall load index and estimating peripheral resistance, we acknowledge the difficulty in obtaining in vivo measures of regional mechanics and hemodynamic data in the rat, and we specifically avoid the implication that we can accurately assess either variable. We also did not evaluate the entire Frank-Starling relation using full pressure volume curves.

Postmortem changes, fixation and processing are known to cause dimensional alterations in tissues. It was assumed that these changes should affect all hearts in a similar manner. Thus, examination of general similarities and relative differences between the groups should be valid. Histologic measures in this study did not include cell length and cell number, both affected by remodeling (40) and GH administration.

Clinical implications. Considering that LV dysfunction due to ischemic heart disease is the leading cause of congestive heart failure in the United States, and that few effective drugs are available, our findings may have substantial clinical implications. Among the most appealing aspects of GH is its ability

to beneficially affect several of the pathologic compensatory processes that follow large MI, especially the early remodeling process, something few treatment strategies designed to reduce postinfarction heart failure are able to modify. Thus, in strategies to reduce ischemia or heart failure, GH may occupy a unique niche temporally located between efforts to reduce direct injury related to infarct size (thrombolysis, primary angioplasty) and longer term maintenance therapies that modulate neurohumoral, hemodynamic and arrhythmic consequences of infarction, such as angiotensin-converting enzyme inhibitors and beta-blockers. Although it is tempting to postulate that GH may play a "preventive" rather than a palliative role in the treatment of postinfarction heart failure, further definition of its effects and benefits will be necessary.

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